

FIG. 1

The nucleotide coding sequence (SEQ ID NO:1) and amino acid sequence (SEQ ID NO:2) of bovine lysozyme

```
atg aag gct ctc gtt att ctg ggg ttt ctc ttc ctt tct gtc gct
M  K  A  L  V  I  L  G  F  L  F  L  S  V  A

gtc caa ggc aag gtc ttt gag aga tgt gag ctt gcc aga act ctg
V  Q  G  K  V  F  E  R  C  E  L  A  R  T  L

aag aaa ctt gga ctg gac ggc tat aag gga gtc agc ctg gca aac
K  K  L  G  L  D  G  Y  K  G  V  S  L  A  N

tggttg tgg acc aaa tgg gaa agc agt tat aac aca aaa gct
W  L  C  L  T  K  W  E  S  S  Y  N  T  K  A

aca aac tac aat cct agc agt gaa agc act gat tat ggg ata ttt
T  N  Y  N  P  S  S  E  S  T  D  Y  G  I  F

cag atc aac agc aaa tgg tgg tgt aat gat ggc aaa acc cct aat
Q  I  N  S  K  W  W  C  N  D  G  K  T  P  N

gca gtt gac ggc tgt cat gta tcc tgc agc gaa tta atg gaa aat
A  V  D  G  C  H  V  S  C  S  E  L  M  E  N

gac atc gct aaa gct gta gcg tgt gca aag cat att gtc agt gag
D  I  A  K  A  V  A  C  A  K  H  I  V  S  E

caa ggc att aca gcc tgg gtg gca tgg aaa agt cat tgt cga gac
Q  G  I  T  A  W  V  A  W  K  S  H  C  R  D

cat gac gtc agc agt tac gtt gag ggt tgc acc ctg taa
H  D  V  S  S  Y  V  E  G  C  T  L  *
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FIG. 2 (sheet 1 of 4)

Nucleotide sequence of the plasmid p1044-BoLys

(extends from nucleotides 5767 – 6211 of the viral vector; the sequence encoding bovine lysozyme, including the stop codon, is inserted as a PacI-XhoI fragment and is shown in lower case letters, underscored)

GTAATTTTAC AACAAATTACC AACAAACAACA AACAAACAGAC AACATTACAA TTACTATTTA CAATTACAAT GGCATACACA CAGACAGCTA
 CCACATCAGC TTGTCTGGAC ACTGTCCGAG GAAACAACCTC CTGTGTCAT GATCTAGCAA AGCGTCGTCT TTACGACACA GCGGTTGAAG
 AGTTTAAACG TCGTGACCG AGGCCAAGG TGAACCTTTC AAAAGTAATA AGCAGGAGC AGACGCTTAT TGCTACCCGG GCGTATCCAG
 AATTCCAAAT TACATTTTAT AACACGCAA ATGCCGTGCA TTCCGCTTGA GGTGGATTGC GATCTTTAGA ACTGGAATAT CTGATGATGC
 AAATCCCTA CGGATCATG ACTTATGACA TAGCGGGAA TTTTGCACTG CATCTGTTCA AGGACGAGC ATATGTACAC TGCTGCATGC
 CCAACCTGGA CGTTCGAGC ATCATGCGC ACAGAGGCA GAAAGACAGT ATTGAACAT ACCTTCTAG GCTAGAGAGA GGGGGGAAA
 CAGTCCCAA CTTCACAAAG GAAGCATTTG ACAGATACG AAAAACTCCT GAAAGCGCTG TCTGTACAA TACTTTCCAG ACATGCGAAC
 ATCAGCCGAT GCAGCAATCA GCGAGAGTGT ATGCCATTGC GCTACACAGC ATATATGACA TACCAGCCGA TGAGTTCGG GCGCACTCT
 TGAGGAAAAA TGTCGCGGAT TGCTATGCCG CTCTCCACTT CTCCGAGAAC CTGCTTCTTG AAGATTCTAG TTAATTACTG TCATAGTTAT ACCTGGTTTT
 ACGCGTGTGT TTAAGTATGT GTGCAAAACT TACTTCCCGG CCTCTAATAG AGAGTTTAC ATGAAGGAGT TTTTAGTCAC CAGAGTTAAT ACCTGGTTTT
 GTAAAGTTTC TAGAATAGAT ACTTTTCTTT TGTAACAAG TGTCGCAACG CGAGAGAACT CTCCCTGAGG ATTCATCATC AGTCAATTAC TGGTTTCCCA
 ACGCATGGCA TTACAAAAAG ACTCTTGCAA TGTCGCAACG CGAGAGAACT CTCCCTGAGG ATTCATCATC AGTCAATTAC TGGTTTCCCA
 AAATGAGGGA TATGGTCATC GTACCATTTAT TCGACATTTT TTTGGAGACT AGTAAGAGGA AGTAAGAGGA CCGCAAGGA ATGTTTGTG CTTCTGTGAT
 TCGTGTTCAC AGTGCTTAAC CACATTCGAA CATACGAGC GAAAGCTCTT ACATACGCAA ATGTTTGTG CTTCTGTGAT TCGATTCGAT
 CGAGGGTAAT CATTAACGGT GTGACAGCGA GGTCCGAATG GGATGTGGAC AAATCTTGT TACAATCTT GTCCATGACG TTTTACCTGC
 ATACTAAGCT TGCCGTTCTA AAGGATGACT TACTGATTAG CAAGTTTAGT CTCGGTTCTG AAACGGTGTG CCAGCATGTG TGGGATGAGA
 TTTTCGCTGC GTTTGGGAAC GCATTTTCCCT CCGTGAAAGA GAGGCTCTTG AACAGGAACT TATACAGAT TTATCAGAGT GGCAGCGAC GCATTAGAGA
 TCAGGGTGC TGATCTATAT GTGACCTTCC ACGACAGATT AGTGACTGAG TACAGGCTT TACAGGCTT CTGTGGACAT GCTGCGCTT GACATTAGGA
 AGAAGATGGA AGAAACGGA GTGATGTACA ATGCACCTTC AGAATTATCG GTGTTAAGG AGTCTGACAA ATTCGATGTT GATGTTTTT
 CCCAGATG CCAATCTTG GAAGTTGACC CAATGACGGC AGCGAAGGTT ATAGTCGCGG TCATGAGCAA TGAGAGCGGT CTGACTCTCA
 CATTGAACG ACCTACTGAG GCGAATGTTG CGTAGCTTT ACAGGATCAA GAGAAGGCTT CAGAAGTGC CAGAGGAGT ATTGGTAGT ACCTCAAGAG
 AAGTTGAAGA ACCGTCCATG AAGGTTTCTG TGCCAGAGG AGAGTTACAA TTAGTGGTC TTGCTGGAG TCATCCGGA TCCTCTATT
 CTAAGAACGA GGAGATAGAG TCCTTAGAGC AGTTTCATAT GGCACGGA GATTCTGTTAA GATTCGTTAA TTGTAAGCA GATGAGCTCG ATTGTGTACA
 CCGGTCGGAT TAAAGTTTCA CAAATGAAAA ACTTTATCGA TAGCCTGGTA GCATCACTAT CTGCTCGGT GTCGAATCTC GTCAAGATCC
 TCAAGATAC AGCTGCTATT GACCTTGAAA CCCGTCAAAA GTTTGGAGTC TTGGATGTTG CATCTAGGAA GTGGTTAATC AAACCAACGG

FIG. 2 (sheet 2 of 4)

CCAAGAGTCA	TGCATGGGGT	GTTGTTGAAA	CCCACGGGAG	GAAGTATCAT	GTGGCGCTTT	TGGAATATGA	TGAGCAGGGT	GTGGTGACAT
GCGATGATTG	GAGAAAGAGTA	GCTGTTAGCT	CTGAGTCTGT	TGTTTATTC	GACATGGCGA	AACTCAGAACT	TCTGCGCAGA	CTGCTTCGAA
ACGGAGAAC	GCAATGTCAGT	AGCGCAAAAG	TTGTTCTTGT	GGACGGAGTT	CCGGCTGTG	GAATAACCAA	AGAAATCTTT	TCCAGGGTTA
ATTTTGATGA	AGATCTAATT	TTAGTACCTG	GGAAGCAAGC	CGCGAAATG	ATCAGAAGAC	GTGCGAATTC	CTCAGGGATT	ATTGTGGCCA
CGAAGGACAA	CGTTAAACC	GTTGATCTTT	TCATGATGAA	TTTTGGGAAA	AGCACACGCT	GTGAGTTCAA	GAGGTTATTC	ATTGATGAAG
GGTTGATGTT	GCATACCTGGT	TGTGTTAATT	TTCTTTGGC	GATGTCATTG	TGCGAAATG	CATATGTTTA	CGGAGACACA	CAGCAGATTTC
CATACATCAA	TAGAGTTTCA	GGATTCCTGT	ACCCGCGCCA	TTTTGCCAAA	TTGGAAGTTG	ACGAGGTGGA	GACACGCAGA	ACTACTCTCC
GTTGTCCAGC	CGATGTCACA	CATTATCTGA	ACAGAGATA	TGAGGGCTTT	GTGATGAGCA	CTTCTTCGGT	TAAAAAGTCT	GTTTCGCAGG
AGATGGTCGG	CGGAGCCGCC	GTGATCAATC	CGATCTCAA	ACCTTGCA	GGCAAGATCC	TGACTTTTAC	CCAATCGGAT	AAAGAAGCTC
TGCTTTCAAG	AGGGTATTC	GATGTTTACA	CTGTGCATGA	AGTGCAAGGC	GAGACATACT	CTGATGTTTC	ACTAGTTAGG	TTAACCCCTA
CACCGGTCTC	CATCATTTGCA	GGAGACAGCC	CACATGTTT	GGTCGCAATG	TCAAGGCACA	CCTGTTCCGT	CAAGTACTAC	ACTGTTGTTA
TGGATCCCTT	AGTTAGTATC	ATTAGAGATC	ATTAGAGATC	TAGTCTGATC	TTGTTAGATA	TGTATAAGGT	CGATGCGAGA	ACACAATAGC
AATTACAGAT	TGACTCGGTG	TTCAAAGGTT	CCAATCTTTT	TGTTGCAGCG	CCAAAGACTG	GTGATATTTT	TGATATGCGAG	TTTTACTATG
ATAAGTGTCT	CCCAGGCAAC	AGCACCATGA	TGAATAATTT	TGATGCTGTT	ACCATGAGGT	TGACTGACAT	TTTCATTGAAT	GTCAAAGATT
GCATATTGGA	TATGTTCTAAG	TCTGTTGCTG	CGCTTAAGGA	TCAATCAAA	CCACTAATAC	CTATGTTACG	AACGGCGGCA	GAATATGCCAC
GCCAGACTGG	ACTATTGGAA	AATTTAGTGG	CGATGATTAA	AAGAAACITTT	AACGCACCCG	AGTTGTTCTGG	CATCATTTGAT	ATTGAAAAATA
CTGCATCTTT	GGTTGTAGAT	AAGTTTCTT	ATAGTTTATTT	GCCTTAAAGAA	AAAAGAAAAAC	CAATAAAAAA	TGTTTCTTTG	TTCAGTAGAG
AGTCTCTCAA	TAGATGGTTA	GAAAAGCAGG	AACAGGTAAC	AATAGGCCAG	CTCGCAGATT	TTGATTTTGT	GGATTTGCCA	GCAGTTGATC
AGTACAGACA	CATGATTAAA	GCACAACCCA	AACAAAAGTT	GGACACTTCA	ATCCAAACCG	AGTACCCGGC	TTTGCAGACG	ATTGTGTACC
ATTCAAAAAA	GATCAATGCA	ATATTCCGCC	CGTTGTTTAG	TGAGCTTACC	AGGCAATTAC	TGGACAGTGT	TGATTCGAGC	AGATTTTGT
TTTTTCACAAG	AAAGACACCA	GCGCAGATTG	AGGATTTCTT	CGGAGATCTC	GACAGTCAATG	TGCCGATGGA	TGCTTTGGAG	CTGGATATAT
CAAAATACGA	CAATCTCAG	AATGAATTCC	ACTGTGCAGT	AGAATACGAG	ATCTGGCGAA	GATTTGGGTTT	CGAAGACTTC	TTGGGAGAAAG
TTTGGAAACA	AGGCATAGA	AAGACCACCC	TCAAGGATTA	TACCCGAGGT	ATAAAAACTT	GCATCTGGTA	TCAAAAGAAAG	AGCGGGGACG
TCACGACGTT	CATTGGAAAC	ACTGTGATCA	TTGCTGCATG	TTTGGCTCCG	ATGCTTCCGA	TGGAGAAAAAT	AATCAAAAGGA	GCCTTTTGGG
GTGACGATAG	TCTGCTGTAC	TTTCCAAAGG	GTTGTGAGTT	TCCGGATGTG	CAACACTCCG	CGAATCTTAT	GTGGAATTTT	GAAGCAAAAC
TGTTTAAAAA	ACAGTATGGA	TACTTTTGGC	GAAGATATGT	AATACATCAC	GACAGAGGAT	GCATTGTGTA	TTACGATCCC	CTAAAGTTGA
TCTCGAAACT	TGGTCTTAAA	CACATCAAGG	ATTGGGAACA	CTTGGAGGAG	TTTCAAGAGG	CTCTTTGTGA	TGTTGCTGTT	TCGTTGAACA
ATTGTGCGTA	TTACACACAG	TTGGACGACG	CTGTATGGGA	GGTTTCATAAG	ACCGCCCTC	CAGGTTCCGT	TGTTTATAAA	AGTCTGGTGA
AGTATTGTGC	TGATAAAGTT	CTTTTGTAGAA	GTTTGTATTAT	AGATGGCTCT	AGTTGTTAAA	GGAAAAGTGA	ATATCAATGA	GTTTATCGAC
CTGACAAAAAT	TGGAGAAGAT	CTTACCGTCG	ATGTTTACCC	CTGTAAAGAG	TGTTATGTGT	TCCAAAGTTG	ATAAAATAAT	GGTTTCAATGAG
AATGAGTCAAT	TGTCAGGGGT	GAACCTTCTT	AAAGGAGTTA	AGCTTATTGA	TAGTGGATAC	GTCTGTTTAG	CCGGTTTGGT	CGTCACGGGC
GAGTGGAACT	TGCCTGACAA	TTGCAGAGGA	GGTGTGAGCG	TGTGTCTGGT	GGACAAAAGG	ATGGAAAAGAG	CCGACGAGGC	CATTCTCGGA

FIG. 2 (sheet 3 of 4)

TCTTACTACA CAGCAGCTGC AAAGAAAAGA TTTCAGTTCA AGTCGTTC CAATTATGCT ATAACCACCC AGGACGCGAT GAAAAACGTC
 TGGCAAGTTT TAGTTAATAT TAGAAATGTG AAGATGTGAG CCGGTTTCTG TCCGCTTTCT CTGGAGTTTG TGTCGGTGTG TATTGTTTAT
 AGAATAATA TAAATTTAGG TTGAGAGAG AAGATTACAA ACCTGAGAGA CCGAGGGCCC ATGGAACCTTA CAGAAGAAAT CGTTGATGAG
 TTTCATGGAAG ATGTCCCTAT GTCGATCAGG CTTGCAAGT TTGATCTCG AACCGGAAAA AAGAGTGATG TCCGCAAAAG GAAAAATAGT
 AGTAGTGATC GGTCAAGTGC GAACAAGAAC TATAGAAATG TTAAGGATTT TGGGGGAATG AGTTTAAAAA AGAATAATTT AATCGATGAT
 GATTCGGAGG CTAATGTGCG CGAATCGGAT TCGTTTTAAA TAGATCTTAC AGTATCACTA CTCCATCTCA GTTCGTGTTT TTGTCATTAA
 TTAATAA
atg aag gct ctc gtt att ctg ggg ttt ctc tct gtc gct gtc caa ggc aag gtc ttt gag aga tgt gag
ctt gcc aga act ctg aag aaa ctt gga ctg gac ggc tat aag gga gtc agc ctg gca aac tgg ttg tgt ttg acc
aaa tgg gaa agc agt tat aac aca aaa gct aca aac tac aat cct agc agt gaa agc act gat tat ggg ata ttt
cag atc aac agc aaa tgg tgg tgt aat gat ggc aaa acc cct aat gca gtt gac ggc tgt cat gta tcc tgc agc
gaa tta atg gaa aat gac atc gct aaa gct gta gca agt cat att gtc agt gag caa ggc att aca gcc
tgg gtg gca tgg aaa agt cat tgt cga gac cat gac gtc agc agt tac gtt gag ggt tgc acc ctg taa
 CTCGAGGGGT AGTCAAGATG CATAATAAT AACGGATTGT GTCCGTAATC ACACGTGGTG CGTACGATAA CGCATAGTGT TTTTCCCTCC
 ACTTAAATCG AAGGTTGTG TCTTGGATCG CGCGGTGCAA ATGTATATGG TTCAATATACA TCCGCAAGCA CGTAATAAAG CGAGGGTTC
 GGTGCGAGGT CGGCTGTGAA ACTCGAAAAG GTTCCGGAAA AAAAAAGA GAGTGGTAGG TAATAGTGT TATAATAAGA AATAATAATA
 TAGTGGTAAG AAAGTTTGA AAGTTGAGGA AATTGAGGAT AATGTAAGTG ATGACGAGTC TATCGCGTCA TCGAGTACGT TTTAATCAAT
 ATGCCTTATA CAATCAACTC TCCGAGCCAA TTTGTTTACT TAAGTTCCGC TTATGCAGAT CCTGTGCAGC TGATCAATCT GTGTACAAAT
 GCATTGGGTA ACCAGTTTCA ACGCAACAA GCTAGGACAA CAGTCCAACA GCAATTTGCG GATGCCCTGA AACCTGTGCC TAGTATGACA
 GTGAGATTTT CTGCATCGGA TTTCTATGTG TATAGATATA ATTGACGCT TGATCCGTTG ATCAGCGCGT TATTAAATAG CTTCGATACT
 AGAAATAGAA TAATAGAGGT TGATAATCAA CCGCACCGA ATACTACTGA AATCGTTAAC GCGACTCAGA GGTAGACGA TCGACTGTA
 GCTATAAGGG CTTCAATCAA TAATTTGGCT AATGAACCTGG TTCTGTGGAAC TGGCATGTTT CACTGAAGAC TTAATAATTCA GGTGGCTGA
 CTTGTCTGGA CCACAACCTC GGCTACTTAG CTATTGTTGT GAGATTTCTT AATATAAAGT CACTGAAGAC CCATGTGATG GTGTACTG
 TACCAAAATC AGCAGTGGTT GTTCGTCCAC TTAATAATAA CGATTGTCT ATCTGGATCC AACAGTTAAA CCATGTGATG GTGTACTG
 TGGTATGGCG TAAACAACG GAAAAGTCCG TGAAGACTTA AATTCAGGG TGGCTGATAC CAAAATCAGC AGTGGTTGTT CGTCCACTTA
 AAAATAACGA TTGTATATC TGGATCCAAC AGTTAAACCA TGTGATGGTG TATACTGTGG TATGGCGTAA AACACCGGAG AGGTTTCAAT
 CCTCCCTTAA CCGCGGGTAG CGGCCAGGT ACCCGGATGT GTTTTCCGGG CTGATGAGTC CGTGAGGACG AAACCTGGCT GCAGGATGC
 AAGCTTGGCG TAATcatggt catAGTGT TTCTGTGTGA AATTGTTATC CGCTCACAAT TCCACACAAC ATACGAGCCG GAAGCATAAA
 GTGTAAAGCC TGGGGTGCCT AATGAGTGAG CTAACCTACA TTAATTGGGT TGGCTCACT GCGCGCTTTC CAGTCGGGAA ACCTGTCTGTG
 CCAGCTGCAT TAATGAATCG GCCAACGCGC GGGGAGAGGC GGTTTGGGTA TTCCGCTTCC TCGCTCACTG ACTCGCTGCG

CGACGGTTGTA AAACGACGGC CAGTGAATTC AAGCTTAATA CGACTCACTA

5' 3'

126K 183K 30K hcp

Replicase subunits SP-E SP-1 SP-2

Fig. 3.

10-20% Tris-Glycine SDS PAGE gel

1. Marker
2. (+) BoLys - 1 μ g
3. (+) BoLys - 2 μ g
4. (+) BoLys - 5 μ g
5. Nb-1 GJ - 2 μ l
6. Nb-2 GJ - 2 μ l
7. Nb-3 GJ - 2 μ l

20 kDa
14 kDa

TMV coat protein
bolys

Fig. 4

14% Tris-Glycine SDS-PAGE gel

1. Marker
2. (+) Hen EW lys 5 μ g
3. (+) BoLys - 1 μ g
4. (+) Boys - 2 μ g
5. (+) BoLys - 3.5 μ g
6. (+) BoLys - 5 μ g
7. (+) BoLys - 7 μ g
8. 1051500 IF crude - 1 μ l
9. 1051500 IF crude - 5 μ l
10. 1051100 IF crude - 1 μ l
11. 1051100 IF crude - 5 μ l
12. Marker 12

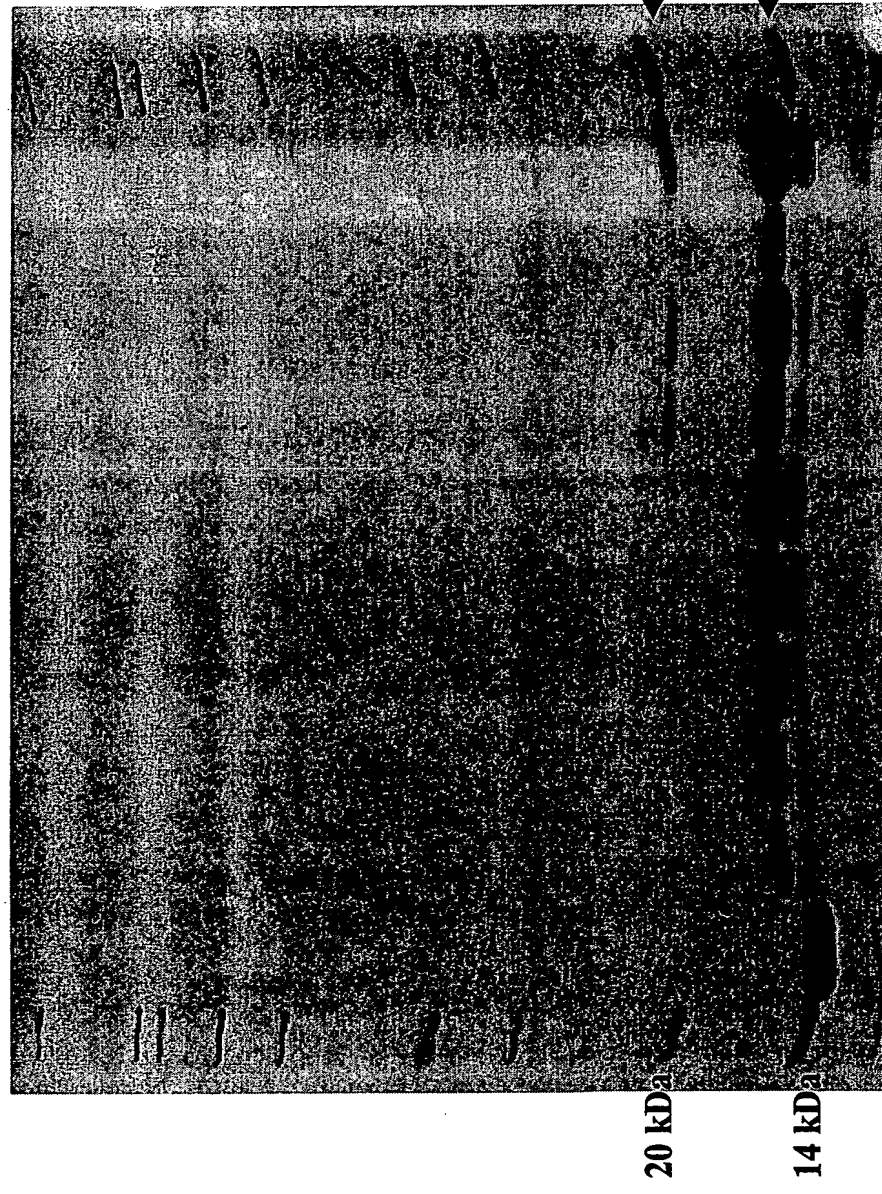


Fig. 5

2025 FEB 26 10:25:00

Laser : 2350
Scans Averaged: 62
Pressure: 1.07e-07
Low Mass Gate: 1000.0
Timed Ion Selector: 24.9 OFF
Negative Ions: OFF
Collected: 4/3/2000 5:13 PM

Method: HCD-60K
Mode: Linear
Accelerating Voltage: 25000
Grid Voltage: 90.000 %
Guide Wire Voltage: 0.100 %
Delay: 300 ON
Sample: 44

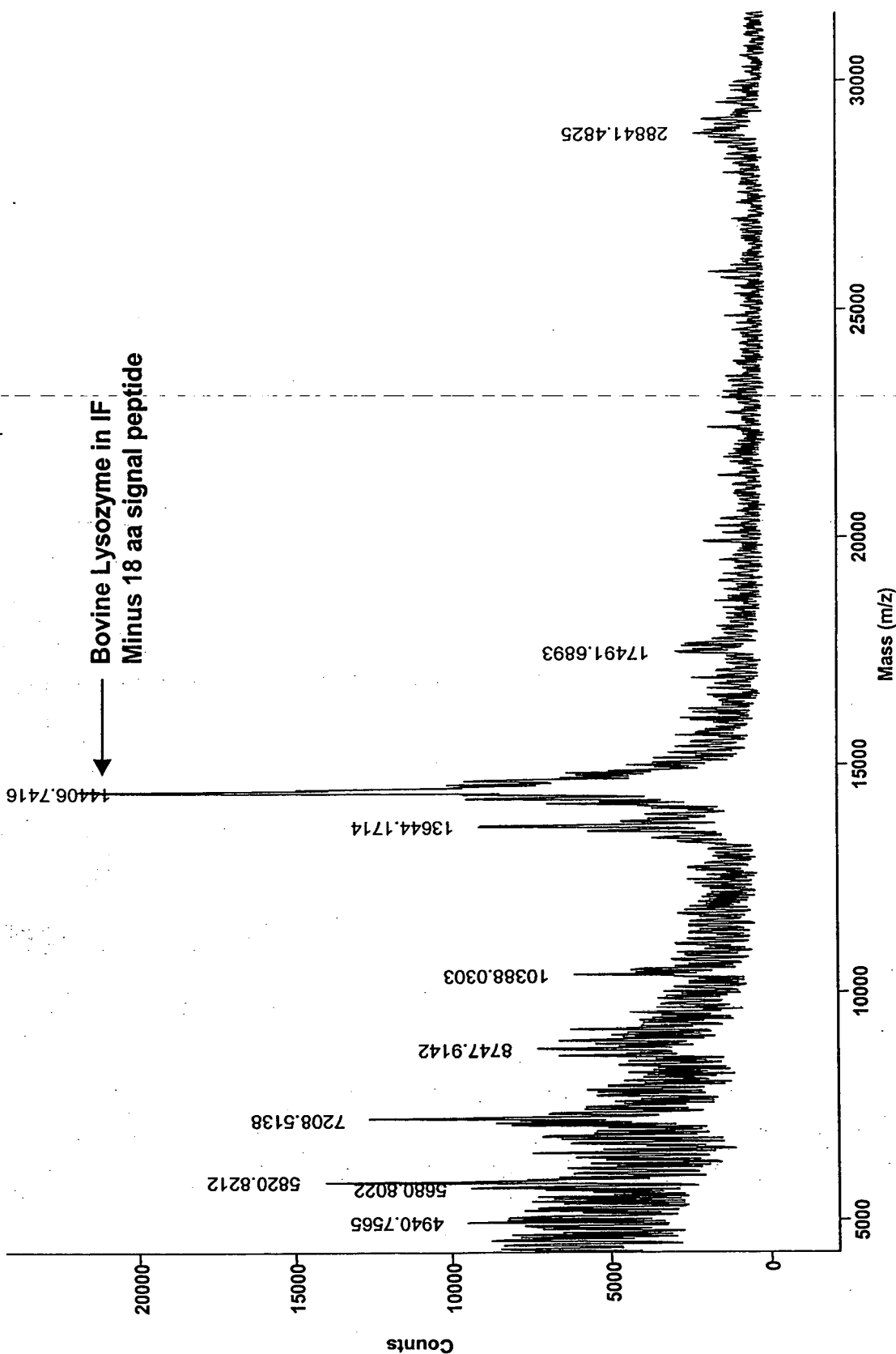


Fig. 6

10=9=00_(2) Chart 1

3K vs. Standard (Turbidimetric)

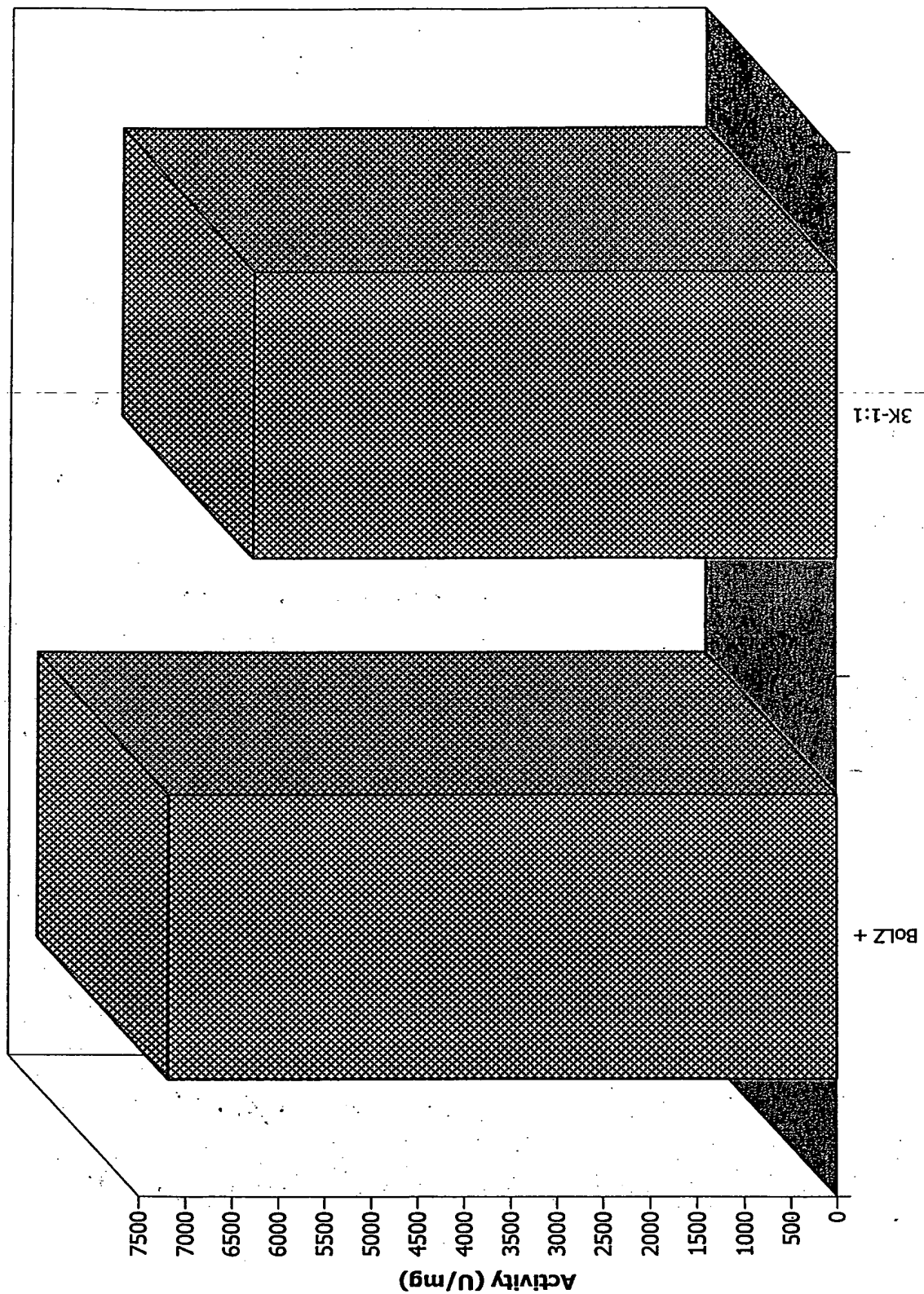


Fig. 7

042800 IF 041800 IF BoLZ Control

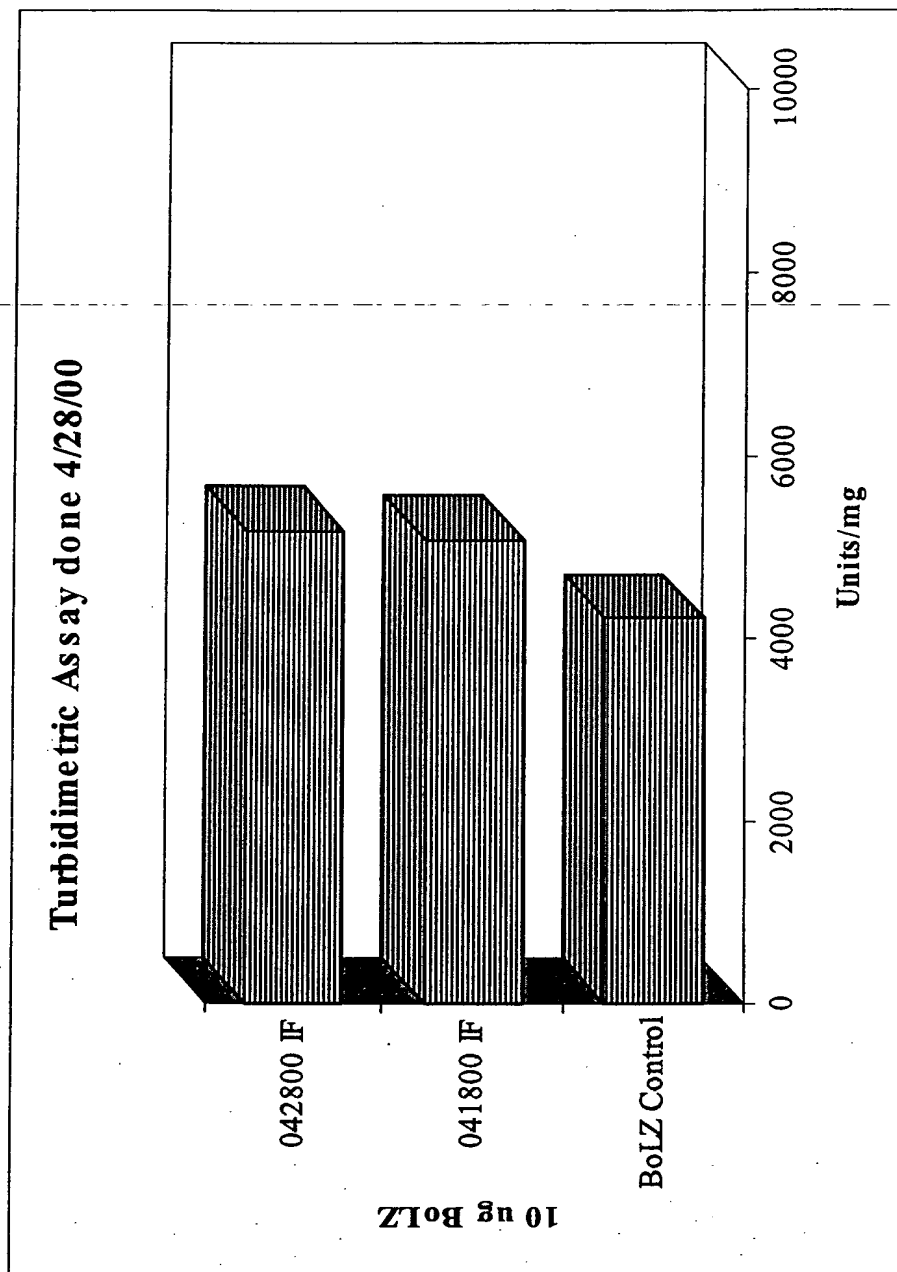


Fig. 8

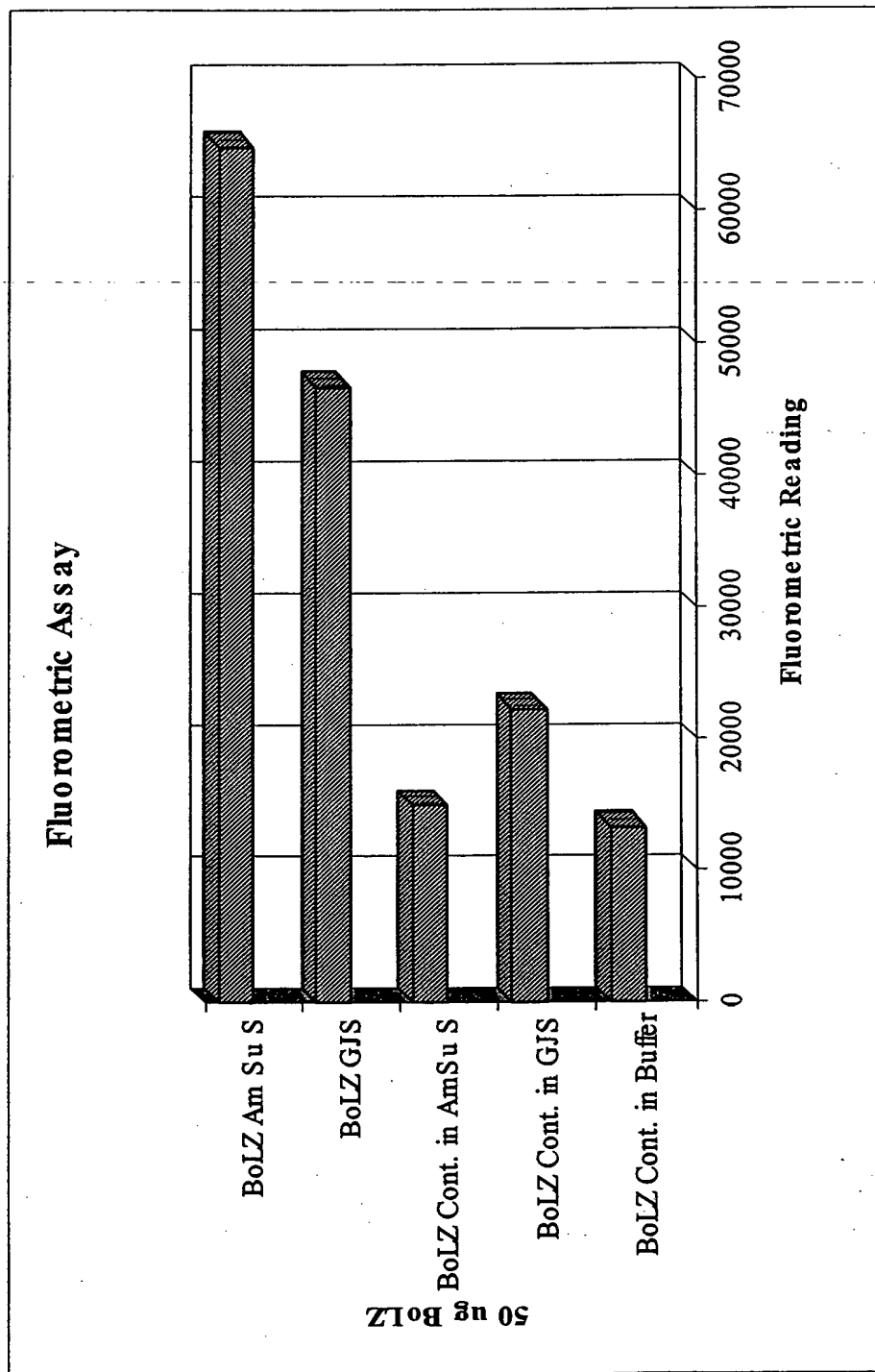


Fig. 9

